

AN IMPROVED METHOD FOR THE DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO EXTRACTS¹

By O. M. SHEDD²

Chemist, Kentucky Agricultural Experiment Station

HISTORICAL REVIEW

The two methods which are now more generally used in this country for the estimation of nicotine in tobacco and tobacco extracts are those of Kissling and the silicotungstic acid method as perfected by Chapin.³ They are the only ones for this determination which have been adopted as official by the Association of Official Agricultural Chemists.⁴ It is of interest to note in this connection that while Chapin's procedure for extracts was adopted by the association, a material change appears in the association method. This change is in the amount and manner of adding the alkali preparatory to the distillation; it is opportune, even if made unwittingly, because it tends to increase the accuracy of the method when applied to tobacco. In this connection it is important that a sufficient quantity of alkali be added in the distillation to liberate combined nicotine. The association method should emphasize this point more clearly, since it is possible in following their directions in the distillation to have the solution test alkaline due to free nicotine and yet an insufficient quantity of alkali may have been added to liberate that in combination.

Chapin describes the various procedures which have been proposed for the estimation of nicotine and the defects he found in their application, so it is not necessary to mention them here. Unfortunately, however, Chapin, in his work on the silicotungstic acid method, did not include tobacco in his studies, otherwise he probably would have found that errors may often occur in the use of this method on tobacco, as will be mentioned later. The method as outlined by him was proposed for tobacco extracts and when so applied in the present work has been found to be more satisfactory than for tobacco itself.

While it is generally recognized that the Kissling method will give good results in the hands of an experienced analyst, provided no interfering substances are present in the determination, it is nevertheless, conceded that the silicotungstic acid method will probably give more reliable and concordant results in ordinary use.

After the work reported in this paper was practically completed, the writer learned for the first time, through a mutual acquaintance, that a similar method had been published by Rasmussen,⁵ in Denmark. As no reference to it could be found in the literature at hand, a copy was obtained

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³ CHAPIN, R. M. THE DETERMINATION OF NICOTINE IN NICOTINE SOLUTIONS AND TOBACCO EXTRACTS. *In* U. S. Dept. Agr. Bur. Anim. Indus. Bul. 133, p. 21. 1911.

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919, p. 65-66. Washington, D. C. 1920.

⁵ RASMUSSEN, HANS BAGGESGAARD. OM BESTEMMELSE AF NIKOTIN. *In* K. Danske Vidensk. Selsk. Skr. Naturvidensk. og Math. Afd., Ser. 8, Bd. 1, p. 66. 1916.

and it was found that while Rasmussen's method is somewhat similar in principle, it is entirely different in technic from the one described in this paper. As no determinations by the former method have been made here, its accuracy is not known. Certain features of it, however, are mentioned for comparison with the writer's procedure.

The extraction of nicotine from the tobacco to which alcoholic soda has been previously added is carried on in a flask containing a mixture of equal parts of ethyl ether and petroleum ether, by frequent shaking for 5 hours. The solvent is then filtered from the tobacco through a folded filter covered with a glass plate. An aliquot is taken for acid extraction. With extracts, 3 or 4 gm. of the sample are placed in a 100 cc. flask together with 5 cc. concentrated caustic soda, 5 cc. water, and 50 cc. of the above mixed ethers. The mixture is allowed to stand 4 or 5 hours with frequent shaking, then 25 cc. of the solvent is drawn off and treated as before. In both cases the nicotine is precipitated with silicotungstic acid. Doubts might be raised as to the complete extraction of the nicotine, especially from extracts handled in the manner indicated, as compared with a Soxhlet extraction of the sample as prepared for analysis here. Furthermore, errors may arise due to evaporation when the solvents are filtered and aliquots withdrawn in the manner described. Finally, no provision is made for the prevention of emulsions often obtained in the acid washing of the ether extract of some samples and without which no method of this character is practical.

EXPERIMENTAL WORK

The writer, having occasion to determine the nicotine content of a large number of various grades and varieties of tobacco grown in Kentucky, found that it would be very convenient to have a method which would be more rapid than either of the official methods and equally accurate. As several determinations were to be made at the same time and facilities for making simultaneous steam distillations were not at hand, it was desirable to have a method that would, if possible, eliminate such distillations. A steam distillation of nicotine is objectionable inasmuch as it is often a long, tedious procedure that requires careful attention in order that the boiling liquid shall be maintained at a low volume to remove all nicotine.

The writer's previous experience with the Kissling method has shown that there are two principal errors to be avoided: First, the presence of ammonia in the nicotine distillate, due to its incomplete separation from the ether extract of the sample, and, second, the possible loss of nicotine in the evaporation of this ether extract previous to its distillation. The first error will, of course, give high results, due to the ammonia being titrated and calculated as nicotine, whereas the other causes low results; consequently, satisfactory figures obtained by the method may be sometimes the result of a balancing of errors. On the other hand, experience with the silicotungstic acid method in this work has shown that while it is decidedly preferable to the Kissling, nevertheless certain points in its use have to be carefully supervised in order to obtain satisfactory results. The writer's experiments show that this method as outlined by Chapin gives more satisfactory results on nicotine extracts than on tobacco. The reasons for this will be mentioned later.

Experience here has shown that the distillation as prescribed in the silicotungstic acid method, even though carried on under proper condi-

tions, is sometimes a long, tedious procedure; and, if not so conducted, will often give inaccurate results on tobacco. Accordingly, it was thought possible to substitute an ether extraction of the sample for the steam distillation of it in the silicotungstic acid method, in order to obtain the nicotine for precipitation. Afterwards, the nicotine could be extracted from the ether by acid and determined in the manner prescribed in this method. Accordingly, considerable work has been carried on with this in view, and, after many preliminary determinations, a method has been devised which has usually been found to be more rapid than either of the official methods and, with some samples at least, gives more accurate results than the silicotungstic acid method unless the latter is carried on under carefully controlled conditions. It combines the good features of both official methods and eliminates an undesirable one common to both, namely, the steam distillation. The method is as follows:

REAGENTS

Alcoholic sodium hydroxid solution: Dissolve 6 gm. of sodium hydroxid in 40 cc. of water and 60 cc. of 90 per cent alcohol.

Silicotungstic acid solution: Prepare a 12 per cent solution of the silicotungstic acid having the formula $4\text{H}_2\text{O}$, SiO_2 , 12WO_3 , $22\text{H}_2\text{O}$. (There are several silicotungstic acids. The acids $4\text{H}_2\text{O}$, SiO_2 , 10WO_3 , $3\text{H}_2\text{O}$ and $4\text{H}_2\text{O}$, SiO_2 , 12WO_3 , $20\text{H}_2\text{O}$, do not give crystalline precipitates with nicotine and should not be used.)

Ethyl ether of quality equal to U. S. P. concentrated.

Dilute hydrochloric acids (1 to 4) and (1 to 1,000).

DETERMINATION

Weigh 1 gm. of tobacco extract (high grade 40 per cent) or more of extracts containing less nicotine; or 5 gm. of finely powdered tobacco which has been previously dried at room temperature or slightly above, to permit powdering, and put into a beaker or, preferably, a porcelain dish. Add 2.5 cc. of the alcoholic sodium hydroxid to the tobacco powder or about 5 cc. to the extract; it is necessary that enough alcoholic sodium hydroxid be added to liberate the nicotine. Follow in the case of extracts with a sufficient quantity of pure powdered calcium carbonate to form a moist but not lumpy mass. Mix thoroughly with a pestle or spatula, transfer to a suitable container in a Soxhlet extractor and exhaust for about 5 hours with ether. (Probably another solvent could be substituted for this purpose.) It is important that the temperature of the cooling water during the extraction should not be much over 20°C ., and sufficient solvent should be used in order that its volume in the extraction flask should never be less than about 25 cc. After the extraction is made, the exhausted sample is removed and the excess of ether recovered, but the volume of ether extract should not be permitted to go below the above minimum or more if necessary to keep the extractive matter in solution at ordinary temperature. (If the temperature of the cooling water is too high and the volume of solvent in the flask becomes too low during the extraction, there may be a small loss of nicotine from samples of high percentage.)

The ether extract is transferred to a separatory funnel, preferably with a short stem, the extraction flask washed with a little ether and finally about twice with hydrochloric acid (1 to 4) to remove all nicotine.

The extract is shaken with four to six portions (10 cc. each) of cold dilute hydrochloric acid (1 to 4). If an emulsion forms during the successive extractions with the acid, this can be prevented by adding a small amount of 95 per cent alcohol, about 1 cc. being sufficient for each separate extraction. (When it is found necessary to use alcohol, it is essential that it be added after the shaking has been completed. Further shaking should be avoided as much as possible, as one or two inversions of the funnel will break the emulsion. A minimum amount of alcohol and shaking are best, since it has been found that when alcohol is added along with the acid, followed by vigorous shaking, it has some solvent action on extraneous material that precipitates with the reagent. Consequently, alcohol should not be used unless necessary, and only in the manner described.) The successive extractions are run through a funnel into a 100 cc. graduated flask. A small ball of fine glass wool may be placed in the neck of the funnel. The extraction should be continued until a few drops of the acid solution give no turbidity with the reagent. The funnel is carefully washed with water and the volume made to 100 cc. An aliquot corresponding to about 0.25 gm. or more of extract or 1 to 2 gm. of tobacco, depending on the amount of nicotine present, is placed in a beaker and diluted to 400 to 500 cc. volume with distilled water. A sufficient quantity of dilute hydrochloric acid (1 to 4) is then added so as to have at least 3 cc. or more present, to each 100 cc. of liquid, including the quantity of acid contained in the aliquot. At this point, the solution should test distinctly acid with a few drops of methyl orange; if not acid, more should be added. There is then added, with constant stirring, 1 cc. of the silicotungstic acid reagent for each 0.01 gm. of nicotine supposed to be present. Stir thoroughly for about 3 to 5 minutes or until the precipitate is crystalline and settles quickly. To insure an excess of the reagent, a few drops are added to the clear supernatant liquid and the absence of any precipitate noted. The solution is allowed to stand at least 18 or preferably about 24 hours. The precipitate is filtered on an ashless filter (C. S. & S. No. 590 or similar quality) and preferably through a Hirsch or Buchner funnel by decanting first the clear liquid and testing a few drops of it with a little nicotine solution to insure an excess of reagent. By decanting practically all the clear solution the filtration is more quickly made. (In case of very small precipitates, the addition of a small amount of ashless filter paper pulp to the precipitate at the time it is to be filtered will insure a clear filtrate.) When the precipitate is transferred to the filter, if the filtrate should be turbid, it is filtered again until clear. If, at the beginning, the precipitate is made crystalline by proper stirring, the filtration is made quickly and without trouble. The precipitate is thoroughly washed with cold dilute hydrochloric acid (1 to 1,000). Transfer the precipitate, without removing from the paper, to a weighed platinum crucible, dry carefully at low heat until the paper is charred, then ignite over a Bunsen burner until all carbon is eliminated. Finally, heat over a Teclu or Meker burner for not over 10 minutes, or over a moderate blast for about 5 minutes. It is not advisable to prolong the final heating beyond the time mentioned, because of a very small but continued loss at this temperature. The weight of the residue multiplied by 0.114 gives the weight of nicotine present in the aliquot used.

In the development of this method, the following experiments were made to test important features of the work:

ABSENCE OF FOREIGN MATERIAL, IN THE ACID SOLUTION OBTAINED FROM THE ETHER EXTRACT WHICH MIGHT PRECIPITATE WITH THE REAGENT

As experiments had shown that such material would more likely be obtained from tobacco than from extracts, aliquots of the same acid solution of tobacco were compared. One aliquot was distilled and determined by the silicotungstic acid method while the other aliquot was handled at this stage as described in the method above. The results are given in Table I.

TABLE I.—Percentage of nicotine in tobacco (moisture free)

No.	Chapin's silicotungstic acid method.	Improved method.
61422	1. 29	1. 33
61423	2. 17	2. 23
61426	0. 70	0. 75
61436	7. 26	7. 30
61437	5. 47	5. 51
Average	3. 38	3. 42

It is apparent from Table I that in the acid solution obtained from the ether extract of these samples, no foreign material is present which might precipitate with the reagent and materially affect the results, unless it is volatile with steam.

THE AMOUNT OF ALCOHOL USED IN THE MANNER PRESCRIBED IN THE METHOD APPARENTLY DOES NOT HAVE ANY APPRECIABLE DETRIMENTAL EFFECT

Duplicate determinations were made on different tobaccos, in one of which alcohol was used as prescribed, while in the other it was omitted. The results are given in Table II.

TABLE II.—Percentage of nicotine in tobacco (moisture free)

No.	Alcohol used.	No alcohol used.
80236	6. 94	6. 80
80237	5. 98	6. 02
80238	6. 35	6. 30
80239	5. 72	5. 82
80273	2. 98	2. 87
80274	2. 10	2. 05
80275	1. 79	1. 77
80276	0. 97	0. 9
80382	7. 77	7. 7
80383	7. 04	6. 94
80384	5. 78	5. 70
Average	4. 86	4. 82

Apparently alcohol used in the quantity and manner prescribed in the method has very little, if any, effect on the results. The use of alcohol would be more likely to affect the results in the analysis of tobacco than in that of extracts. Experience has shown that it is not usually required for extracts and is not always necessary for tobacco.

Alcohol has been found, however, to have a solvent action for extraneous matter contained in the ether extract of some tobaccos if it is added with the acid and thoroughly shaken, as is done in the acid extraction. For example, two of the samples in Table II, when alcohol was used in this manner, gave the following results: No. 80236 = 7.37 per cent and No. 80237 = 6.32 per cent nicotine. On the other hand, it has been repeatedly found that when as much as 5 cc. alcohol is added to an aliquot after the acid extraction is made, identical results are obtained as compared with another aliquot containing no alcohol. However, it should not be used except where it is necessary and then only in the manner prescribed.

When a comparison was made of this method with Chapin's silicotungstic acid method, it was found that higher, and in some samples of tobacco abnormally higher, results were obtained by the latter method. Much better agreement, however, was shown with extracts. Steam distillations showed that whereas not over 750 cc. of distillate was usually sufficient to obtain the nicotine from extracts, a much larger volume was generally required for tobacco, notwithstanding the fact that a smaller amount of nicotine might be present in the larger distillate. Again, it was found essential that the solution in the distilling flask should be maintained at about 15 to 25 cc. in order to distill over the nicotine in a minimum volume of distillate. If the volume of liquid greatly exceeds this extreme difficulty is met with in distilling the nicotine, especially from tobacco. For example, tests with negative results have been made for nicotine in a distillate coming from a comparatively large solution, although further concentration to the optimum volume showed its presence. Another interesting fact observed in the steam distillation was that the nicotine results obtained by its use, especially with tobacco, were largely influenced by the amount of alkali used in the distillation. For instance, it was found that where 2 gm. of sodium hydroxid was used in the distillation higher results were always obtained than when smaller amounts were employed, despite the fact that in the former case the nicotine usually came over in a smaller volume of distillate. In all distillations the liquid in the distilling flask was alkaline, and sufficient alkali was present to liberate combined nicotine. It has been shown in this connection that the larger amount of alkali liberates some volatile compound other than nicotine which comes over in the distillate and is precipitated by the reagent. This substance occurs in variable quantity in tobacco, and a distillate containing it generally has some odor and color. The compound does not give the characteristic test for nicotine, but forms with silicotungstic acid a dirty white precipitate which becomes pinkish on standing. Where this substance occurs in appreciable quantity it can be obtained by distilling with an excess of alkali (2 gm.) the exhausted tobacco obtained from the ether extraction, or, after a weak alkaline distillation is completed, a further addition of alkali up to the above amount and continued distillation will show its presence. There is no doubt but that the excess of alkali and heat used in the distillation acts to a greater or less extent on the tobacco residue

contained in the small volume necessary to liberate the nicotine, and forms a volatile product or products which are found in the distillate, precipitate with the reagent and affect the results. Further work will be done to determine, if possible, the nature of this reaction.

The following precautions are therefore necessary in the distillation: (1) Use the minimum amount of alkali necessary to liberate all the nicotine; (2) keep the volume of liquid in the flask at about 15 to 25 cc. to facilitate the distillation of the nicotine; (3) continue the distillation until a few cubic centimeters of the distillate shows no opalescence when treated with a drop of dilute hydrochloric acid and a drop of the silicotungstic acid. Under such conditions, however, a large volume of distillate will sometimes be obtained, as shown in Table III.

TABLE III.—Percentage of nicotine in tobacco (moisture free); effect of different amounts of NaOH in distillation of 5 gm. sample on the volume of distillate necessary to liberate the nicotine.

No.	2 gm. NaOH.	Volume of distillate.	Final test for nicotine.	Less than 2 gm. NaOH.	Volume of distillate.	Final test for nicotine.
61469	7.55	900	Absent.....	¹ 7.38 ¹ 7.29 ² 6.90	1,500 1,500 2,900	Absent. Do. Do.
61537	6.33	900do.....	² 5.67 5.98	1,800 2,400	Present. Absent.
80073	7.85 7.83	1,800 1,800do.....do.....	¹ 6.76 ¹ 6.65	1,000 1,000	Do. Do.
80144	² 7.32 ² 7.51	2,100 2,700	Present. Absent.

¹ NaOH used in distillation, 0.25 gm.

² NaOH used in distillation, 0.15 gm.

DIRECT PRECIPITATION OF NICOTINE

It was thought that it might be possible with some extracts, provided no interfering substances were present, to make a direct precipitation of the nicotine and thereby eliminate the distillation and ether extraction necessary in the other methods. Experiments were made, therefore, by taking a weighed sample of extract, diluting it to a definite volume and directly precipitating the nicotine in an aliquot with the reagent in the manner prescribed in the improved method as to volume, acidity, and handling of the precipitate. While the precipitates were often colored, very satisfactory results were obtained on most of the extracts, although precipitable matter in others proved to be detrimental. The results obtained are given in Table V.

This method was also tried on tobacco by making an aqueous or weak alkaline extraction, filtering and using an aliquot as described in the preceding paragraph. It was not found applicable, however, as shown by the determinations below, on two samples from Table III. No. 61469 gave 9.13 per cent nicotine by an alkaline extraction and No. 61537 gave 7.53 per cent by an alkaline and 7.37 per cent nicotine by an aqueous extraction.

The results obtained by the improved method on tobacco and extracts in comparison with Chapin's silicotungstic acid method, together with those found by the latter when variable amounts of alkali were used in the distillation, are given in Tables IV and V.

TABLE IV.—Percentage of nicotine in tobacco (moisture free).

No.	Chapin's silicotungstic acid method.		Improved method.
	2 gm. NaOH used in steam distillation of 5 gm. sample.	Less than 2 gm NaOH used in steam distillation of 5 gm. sample.	
61469	7.55	¹ 7.38	6.99
		¹ 7.29	7.01
		² 6.90	7.00
61537	6.33	¹ 7.19	5.98 6.18
		² 5.98	
80073	7.85	¹ 6.76	6.08
	7.83		6.70 6.88
	7.84	¹ 6.65	6.79
80074	6.37	¹ 6.71	6.01
80076	5.32	¹ 6.19	
80117	3.67	¹ 5.09	4.92
80121	3.97	3.37
80126	2.71	3.68
80129	4.57	2.48
80130	4.50	4.43
80134	1.75	4.24
80144	² 7.51	1.55
80195	² 7.77	7.67
80263	¹ 2.56	7.83
			2.57
			2.58
			2.58

¹ NaOH used in distillation, 0.25 gm.

² NaOH used in distillation, 0.15 gm.

TABLE V.—Percentage of nicotine in tobacco extracts and products

No.	Brand.	Guaranty.	Direct precipitation method.	Chapin's silico-tungstic acid method. ¹	Improved method.
43889 ²	16.42	18.77	17.31 17.44	15.96 16.71
80054	"Nico-Fume".....	40.00	41.60	17.38 40.78	16.34 40.77
80055	"Hammond's Tobacco Extract".....	4.00	4.26	4.27	4.25
80056	"Nikoteen".....	30.00	31.67	30.85	30.78
80064	"Thompson's Rose-Nicotine".....	5.00	5.13	5.18 5.26	4.89 4.89
8006518	.72	5.22 .13	4.89 .13
80066	3.57	4.78	3.70 3.82	4.351 4.351
80356	"Hall's Nicotine Solution".....	40.00	40.43	3.76 40.62	3.51 39.69
80357	"Hall's Nicotine Sulfate".....	40.00	40.15	39.82	39.88
40358	"Hall's Nicotine Fumigator".....	12.50	13.26	13.18
80823	"Black Leaf 40".....	40.00	40.54	40.00	40.13
80824	1.64	2.47 2.50	1.65
80826	94.63 94.70	2.49 93.73 93.75 94.60	93.42 93.60 94.14
80828	40.00	40.61 40.73	94.03 39.74 40.19	93.72 40.39 40.88
80829	"Black Leaf 40".....	40.00	40.67 42.21	39.97 41.87	40.64 42.09

¹ In this method, the following weights of sample were distilled with 2 gm. NaOH unless otherwise indicated:

Samples containing 50 per cent or more of nicotine, 0.6 gm.

Samples containing 30 to 50 per cent of nicotine, 1 gm.

Samples containing 3 to 30 per cent of nicotine, 2 gm.

Samples containing less than 3 per cent of nicotine, 5 gm.

² This extract had been prepared for several years and was supposed to contain 16.42 per cent of nicotine, 2.78 per cent of pyridin and 2.37 per cent of ammonium chlorid. The nicotine guaranty is probably only approximate. The results obtained for nicotine by all methods include any pyridin that may have been present in the washed precipitate.

³ NaOH used in distillation, 0.60 gm.

⁴ Duplicates from same ether extraction.

⁵ NaOH used in distillation, 0.15 gm.

⁶ NaOH used in distillation, 0.24 gm.

⁷ NaOH used in distillation, 0.90 gm.

⁸ NaOH used in distillation, 0.30 gm.

SUMMARY

(1) A method has been devised for the determination of nicotine in tobacco and tobacco extracts which has been found to be as rapid and as accurate as the silicotungstic acid method and more satisfactory on tobacco. The other standard method commonly used for nicotine is the Kissling.

(2) The new procedure eliminates an undesirable feature, namely, the steam distillation, common to both of the above methods.

(3) It has been shown that special precautions should be taken in carrying on the distillation in the silicotungstic acid method. Under proper conditions very good results were usually obtained by its use; otherwise, serious errors were often found when working on tobacco.

(4) A direct precipitation method which obviates a preliminary ether extraction or steam distillation to obtain the nicotine, has also been tried and fairly satisfactory results were obtained on most of the tobacco extracts employed. It was not found, however, to be applicable to tobacco and a few extracts.